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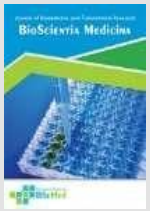

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Neurovascular Inflammation and Oxidative Stress Markers in Chronic Migraine: Is Nitric Oxide the Key Link to Severity?

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A B S T R A C T

Background: Nitric oxide (NO), a ubiquitous signaling molecule, has been implicated in migraine pathophysiology through mechanisms including vasodilation, neurogenic inflammation, and oxidative stress. However, its specific relationship with the clinical severity of chronic migraine required further elucidation. This study aimed to investigate the association between serum NO levels and the severity of chronic migraine in a cohort of female patients. **Methods:** An observational study employing a cross-sectional design was conducted between July 2024 and November 2024 at Neurology Clinics and Community Healthcare Centers in Padang City, Indonesia. Fifty-one female chronic migraineurs, diagnosed according to ICHD-3 criteria, were enrolled using consecutive sampling. Patients with specific comorbidities, pregnancy, breastfeeding, or Medication Overuse Headache (MOH) were excluded. Migraine severity during an ictal phase was assessed using the Migraine Severity Scale (MIGSEV). Venous blood samples were collected during migraine attacks (ictal phase), and serum NO levels were quantified using a colorimetric method. The association between NO levels and MIGSEV scores was analyzed using the Kruskal-Wallis test, followed by post-hoc Mann-Whitney U tests. Statistical significance was set at $p < 0.05$. **Results:** The study included 51 female chronic migraineurs with a median age of 33 years. Migraine severity distribution was: 10 (19.6%) mild, 26 (51.0%) moderate, and 15 (29.4%) severe. The overall median serum NO level was 74.8 nmol/ml (range: 32.20 - 169.15 nmol/ml). Median NO levels demonstrated a positive gradient with increasing migraine severity: mild group 47.31 nmol/ml (range: 34.85 - 67.15), moderate group 88.45 nmol/ml (range: 32.20 - 167.45), and severe group 96.71 nmol/ml (range: 65.45 - 169.15). The Kruskal-Wallis test revealed a statistically significant difference in NO levels across the severity groups ($p < 0.01$). Post-hoc analyses confirmed significant differences between the mild and moderate groups ($p < 0.01$) and between the mild and severe groups ($p < 0.01$). **Conclusion:** This study demonstrated a significant positive association between serum Nitric Oxide levels, measured during the ictal phase, and the severity of chronic migraine in female patients. Higher NO levels were correlated with greater migraine severity, suggesting NO may play a crucial role in the mechanisms underlying migraine intensity and potentially serve as a biomarker reflecting the clinical burden of chronic migraine.

1. Introduction

Migraine represents a substantial global health challenge, recognized as a leading cause of disability worldwide, particularly affecting individuals during their most productive years. This neurological disorder is characterized by recurrent episodes of headache, frequently unilateral and pulsating, and often accompanied by symptoms such as nausea, vomiting, photophobia, and phonophobia. These attacks can severely impair an individual's ability to function in daily life and significantly diminish their overall quality of life. The impact of migraine is underscored by the Global Burden of Disease (GBD) 2019 study, which highlighted the increasing burden

of this condition, noting 87.6 million incident cases globally, a marked rise from the figures reported in 1990. Countries like Indonesia are among those experiencing high incidence rates. Local studies, such as one conducted in Jakarta, have indicated a high prevalence of migraine (45.3%), especially among young women. Migraine exists on a spectrum, ranging from episodic migraine, characterized by less than 15 headache days per month, to chronic migraine (CM), defined by the presence of ≥15 headache days per month for a period exceeding three months, with migrainous features on at least 8 of those days. A concerning proportion of individuals with episodic migraine, estimated at around 2.5% annually, progress to CM, a process known as chronification. This transition is observed more frequently in women and is associated with increased disability and a greater reduction in quality of life. The severity of migraine attacks is a critical factor influencing this burden, with moderate-to-severe attacks showing a strong correlation with poorer quality of life outcomes. Individuals with chronic migraine, in particular, tend to experience more severe attacks compared to those with episodic migraine.[1-3]

The pathophysiology of migraine is complex and not yet fully understood, involving a series of events that originate from neuronal hyperexcitability within the central nervous system. Exposure to various triggers can initiate neurochemical changes, leading to the activation of the trigeminovascular system, a network involving trigeminal afferents that innervate cranial blood vessels. Key phenomena implicated in migraine include Cortical Spreading Depression (CSD), which is a wave of neuronal and glial depolarization followed by a prolonged suppression of activity. CSD is thought to underlie migraine aura and potentially trigger headache mechanisms. The activation of the trigeminovascular system results in the release of vasoactive neuropeptides, such as Calcitonin Gene-Related Peptide (CGRP) and Nitric Oxide (NO), which contribute to vasodilation, plasma protein extravasation, and mast cell degranulation, collectively referred to as neurogenic inflammation. Nitric Oxide (NO), a gaseous signaling molecule synthesized by NO synthases (NOS), plays a multifaceted role in the pathogenesis of migraine. The ability of NO to induce migraine-like headaches was initially demonstrated in experimental studies utilizing NO donors, such as nitroglycerin (NTG). NO exerts its effects through several pathways, including directly causing vasodilation via the activation of soluble guanylate cyclase (sGC) and the subsequent production of cyclic GMP (cGMP) in smooth muscle cells. It also modulates trigeminal nociception, promotes neurogenic inflammation, and contributes to oxidative/nitrosative stress. Evidence suggests that elevated NO levels can increase CGRP production, further amplifying the inflammatory cascade involved in migraine. Furthermore, pharmacological inhibition of NOS or the NO signaling pathway has been shown to attenuate neurogenic inflammation and alleviate headache in both preclinical and clinical settings, supporting the pivotal role of NO in migraine.[4-6]

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, is increasingly recognized as a significant factor in migraine. NO can react with superoxide radicals to form peroxynitrite, a potent oxidant, thereby linking NO signaling directly to oxidative damage mechanisms that may contribute to migraine chronification and severity. Despite the compelling evidence linking NO to migraine mechanisms, studies investigating peripheral NO levels in migraineurs have produced inconsistent results. Some research has reported elevated NO serum or plasma levels in migraine patients, particularly during attacks (ictal phase) or in those with chronic forms of migraine, when compared to controls or individuals with episodic migraine. In contrast, other studies have found no significant differences in NO levels between migraineurs, especially during the interictal phase, and healthy individuals. These discrepancies in findings might be attributed to variations in study populations, such as differences between chronic and episodic migraineurs, or those with and without aura. The timing of sample

collection, whether during the ictal or interictal phase, measurement techniques, and the control for confounding factors that are known to influence NO levels, such as age, BMI, diet, medications, and comorbidities, can also contribute to the inconsistencies. Notably, there has been a scarcity of research specifically examining the direct relationship between peripheral NO levels and the clinical severity of migraine attacks, especially within the context of chronic migraine. Given the established impact of migraine severity on patient quality of life and the proposed roles of NO in driving key pathological processes like neuroinflammation and oxidative stress, understanding this relationship is of significant clinical and pathophysiological relevance.[7-10] Therefore, this study was designed to investigate the association between serum NO levels, measured during the ictal phase, and the severity of migraine attacks, assessed using the MIGSEV scale, in a well-defined cohort of female chronic migraineurs in Padang City, Indonesia.

2. Methods

This investigation employed an observational methodology with a cross-sectional design. The study aimed to capture a snapshot of the relationship between serum Nitric Oxide (NO) levels and the severity of chronic migraine at a single point in time. This design is suitable for exploring associations between variables in a defined population.

The study was conducted from July 2024 to November 2024. The research took place within the Neurology Clinics of referral hospitals and several Community Healthcare Centers (Puskesmas) in Padang City, West Sumatra, Indonesia. Padang City, a major urban center in West Sumatra, provided access to a diverse population of individuals seeking neurological care for headache disorders. The Community Healthcare Centers (Puskesmas) are primary healthcare facilities that serve as the first point of contact for many patients, while the referral hospitals offer specialized neurological services. This multi-site approach enhanced the representativeness of the sample.

Participants were recruited using a consecutive sampling method. This non-probability sampling technique involves enrolling all individuals who meet the inclusion criteria and are available during the study period. Consecutive sampling helps to minimize selection bias by ensuring that all eligible patients have an equal chance of being included in the study.

The inclusion criteria were carefully defined to ensure the selection of an appropriate study population focused on chronic migraine. The primary inclusion criterion was that participants had to be adult women aged 18 years up to the pre-menopausal period. This age range was chosen because migraine disproportionately affects women of reproductive age, and hormonal fluctuations are known to play a significant role in migraine pathophysiology. Additionally, a formal diagnosis of chronic migraine was required. This diagnosis had to be established according to the diagnostic criteria outlined in the International Classification of Headache Disorders, 3rd edition (ICHD-3) beta version (2018). The ICHD-3 criteria are the gold standard for diagnosing headache disorders and ensure consistency and accuracy in migraine classification. All participants were required to provide informed consent prior to enrollment in the study. This ensured that participants were fully aware of the study's purpose, procedures, potential risks, and benefits and that their participation was voluntary. Exclusion criteria were implemented to minimize confounding factors and ensure a homogenous study group focused on chronic migraine. Patients were excluded if they were pregnant or breastfeeding. Pregnancy and breastfeeding involve significant hormonal changes that could influence NO levels and migraine patterns, potentially confounding the results. Individuals with a history of severe mental disorders were also excluded. Severe mental disorders can affect pain perception, stress levels, and overall health, which might indirectly influence migraine and NO levels. Furthermore, patients with significant neurological or systemic comorbidities that could independently influence NO levels or present with

headache symptoms were excluded. These conditions included intracranial tumors, a history of intracranial infections, stroke, significant head trauma, diagnosed hypertension, diabetes mellitus, and other known cardiovascular diseases. These comorbidities were excluded because they can directly or indirectly affect NO production, vascular function, and the experience of headache, potentially obscuring the specific relationship between NO and migraine severity. Patients meeting the criteria for Medication Overuse Headache (MOH) were excluded. MOH is a condition that arises from the overuse of headache medications and can lead to chronic daily headaches, which can be difficult to differentiate from chronic migraine and may also influence NO levels. Individuals with mixed headache types, specifically those with coexisting tension-type headache meeting its own diagnostic criteria, were also excluded. This exclusion criterion was important to isolate the focus on chronic migraine pathophysiology, as tension-type headache has a different underlying mechanism and presentation.

Upon enrollment, several types of data were collected from each participant to provide a comprehensive understanding of their condition and potential influencing factors. Demographic data, including age, marital status, and education level, were collected. Age was recorded in years to allow for continuous analysis. Marital status was categorized (e.g., married, single) to explore potential social and lifestyle influences. Education level was categorized (e.g., primary-high school, diploma-bachelor's degree) to assess socioeconomic factors. Clinical characteristics of migraine were also recorded. These included the presence or absence of aura, a family history of migraine, Body Mass Index (BMI), average monthly frequency of migraine attacks, and the average duration of typical attacks. Aura, a complex of neurological symptoms that can precede or accompany a migraine headache, was recorded as present or absent. Family history of migraine, a significant risk factor for migraine, was recorded as positive or negative. BMI, calculated as weight in kilograms divided by height in meters squared (kg/m^2), was used to assess participants' weight status. The average monthly frequency of migraine attacks was recorded as the number of days per month with migraine headaches. The average duration of typical attacks was recorded in hours.

Migraine severity was quantitatively assessed using the Migraine Severity Scale (MIGSEV). The MIGSEV scale is a validated instrument designed to grade the severity of migraine attacks. It assesses the severity of migraine attacks based on functional impairment and symptom intensity. For this study, the MIGSEV assessment was performed concerning a migraine attack occurring at the time of consultation or within the immediate period (ictal phase). This ensured that migraine severity was assessed during an active migraine attack, when symptoms and functional impairment are most pronounced. Based on the scores obtained from the MIGSEV scale, participants were categorized into three severity groups: Grade 1 (Mild), Grade 2 (Moderate), and Grade 3 (Severe). This categorization allowed for a comparison of NO levels across different levels of migraine severity.

Venous blood samples were collected from each participant during a migraine attack (ictal phase). To ensure consistency and minimize variability, blood samples were collected following standard phlebotomy procedures. Blood was drawn into appropriate collection tubes. Serum was separated from the blood samples by centrifugation, a standard laboratory procedure used to separate blood components based on their density. The separated serum was then used for the quantification of Nitric Oxide (NO) levels.

The concentration of Nitric Oxide (NO) in the serum was determined using a colorimetric assay method. Colorimetric assays are widely used laboratory techniques that measure the concentration of a substance by measuring the intensity of the color produced in a chemical reaction. This method typically relies on the Griess reaction. The Griess reaction is a chemical reaction that quantifies nitrite (NO_2^-), a stable oxidation product of NO. Since nitrite levels are directly related to NO production, measuring nitrite

provides an indirect measure of total NO production. Serum NO levels were expressed in nanomoles per milliliter (nmol/ml). This unit of measurement is a standard way of expressing the concentration of a substance in a liquid.

All collected data were entered and managed using statistical software. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version 26.0. SPSS is a widely used statistical software package that allows researchers to perform a variety of statistical analyses. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study cohort. Descriptive statistics provide a way to organize and summarize data in a meaningful way. Continuous variables, such as age and BMI, were presented as median (minimum-maximum) or mean ± standard deviation (SD), as appropriate based on the data distribution. The median is the middle value in a dataset, while the mean is the average. Standard deviation measures the dispersion or variability of the data around the mean. Categorical variables, including education level, marital status, family history, migraine type, and MIGSEV severity grade, were presented as frequencies and percentages (n, %). Frequencies represent the number of times a particular category occurs, while percentages represent the proportion of times a category occurs relative to the total sample size. Due to the anticipated non-normal distribution of NO serum level data, non-parametric tests were employed for inferential analysis. Non-parametric tests are statistical tests that do not assume that the data follows a normal distribution. Since biological data often do not follow a normal distribution, non-parametric tests are appropriate for analyzing such data. The primary analysis aimed to determine the association between serum NO levels and chronic migraine severity categories (mild, moderate, severe). To compare the median NO serum levels across the three MIGSEV severity groups, the Kruskal-Wallis H test was used. The Kruskal-Wallis H test is a non-parametric equivalent of one-way ANOVA (analysis of variance). It is used to compare the medians of two or more independent groups. If the Kruskal-Wallis test indicated a statistically significant difference ($p < 0.05$), post-hoc analyses were conducted. Post-hoc analyses are additional tests performed after a significant result in an ANOVA or Kruskal-Wallis test to determine which specific groups differ from each other. In this study, the Mann-Whitney U test was used for pairwise comparisons between the severity groups. The Mann-Whitney U test is a non-parametric test used to compare the medians of two independent groups. Pairwise comparisons were made between the mild vs. moderate, mild vs. severe, and moderate vs. severe groups to identify specific group differences. A Bonferroni correction or similar adjustment for multiple comparisons might typically be applied, although this is not explicitly stated in the source document. Multiple comparison corrections are used to reduce the risk of Type I errors (false positives) when performing multiple statistical tests. A p-value less than 0.05 was considered statistically significant for all analyses. The p-value represents the probability of obtaining the observed results if there were no real effect. A p-value less than 0.05 is a conventional threshold for statistical significance, indicating that the results are unlikely to have occurred by chance.

The study protocol was implicitly approved by institutional review boards or ethics committees (Faculty of Medicine Universitas Andalas, Padang, Indonesia). Ethical approval is necessary to ensure that research involving human subjects is conducted ethically and responsibly. The requirement for signed informed consent from all participants indicates that standard ethical principles for research involving human subjects were adhered to throughout the study. These principles include respecting participants' autonomy, ensuring their well-being, and maintaining confidentiality.

3. Results

Table 1 presents a summary of the key demographic and clinical features of the 51 individuals participating in the chronic migraine

study. Regarding demographics, the median age of the participants was 33 years, with ages ranging from 18 to 50 years. The average Body Mass Index (BMI) for the group was 23.19, with a standard deviation of 2.97. In terms of education level, the majority of the participants, 35 out of 51 (68.6%), had attained a Diploma or Bachelor's Degree, while 16 participants (31.4%) had a primary or high school level of education. Looking at marital status, most participants were married, with 34 out of 51 (66.7%) falling into this category, and 17 (33.3%) being single. When considering the clinical migraine features, a significant proportion of the participants reported a family history of migraine, with 38 out of 51 (74.5%) indicating a positive family history, compared to 13 (25.5%) who reported no family history. The predominant migraine type observed in this group was migraine without aura, affecting 43 participants (84.3%), while 8 participants (15.7%) experienced migraine with aura. The median migraine frequency was 16 days per month, with a range from 15 to 24 days. The median duration of a typical migraine attack was 6 hours, with a range from 4 to 12 hours. Finally, regarding migraine severity, participants were categorized into three groups: mild, moderate, and severe. The distribution showed that 10 participants (19.6%) experienced mild migraine, 26 participants (51.0%) experienced moderate migraine, and 15 participants (29.4%) experienced severe migraine.

Table 1. Demographic and clinical characteristics of chronic migraine participants (N=51).

Characteristic	Category / Statistic	Value
Demographics		
Age (years)	Median (Minimum - Maximum)	33 (18 - 50)
Body mass index (BMI) (kg/m²)	Mean ± Standard Deviation (SD)	23.19 ± 2.97
Education level		
	Primary - High School	16 (31.4%)
	Diploma - Bachelor's Degree	35 (68.6%)
Marital status		
	Married	34 (66.7%)
	Single	17 (33.3%)
Clinical migraine features		
Family history of migraine		
	Yes	38 (74.5%)
	No	13 (25.5%)
Migraine type		
	Migraine with Aura	8 (15.7%)
	Migraine without Aura	43 (84.3%)
Migraine frequency (days/month)	Median (Minimum - Maximum)	16 (15 - 24)
Migraine duration (hours/attack)	Median (Minimum - Maximum)	6 (4 - 12)
Migraine severity (MIGSEV)		
	Grade 1 (Mild)	10 (19.6%)
	Grade 2 (Moderate)	26 (51.0%)
	Grade 3 (Severe)	15 (29.4%)

Table 2 presents a comparison of Nitric Oxide (NO) serum levels across different grades of migraine severity, as classified by the MIGSEV score. The table also includes the p-value from the statistical test used to determine if the differences in NO levels between the severity groups are statistically significant. Specifically, the table shows the median NO serum levels (in nmol/ml) and the range (minimum-maximum) for each migraine severity group. For the mild severity group (Grade 1), with 10 participants,

the median NO serum level was 47.31 nmol/ml, with a range from 34.85 to 67.15 nmol/ml. In the moderate severity group (Grade 2), which had 26 participants, the median NO serum level was 88.45 nmol/ml, ranging from 32.20 to 167.45 nmol/ml. For the severe severity group (Grade 3), consisting of 15 participants, the median NO serum level was 96.71 nmol/ml, with a range from 65.45 to 169.15 nmol/ml. The table also indicates that the p-value for the Kruskal-Wallis test was less than 0.01 ($p < 0.01$). The asterisk denotes that the Kruskal-Wallis test was used. This p-value signifies that there is a statistically significant difference in NO serum levels between at least two of the migraine severity groups. In other words, the observed differences in NO levels across the mild, moderate, and severe migraine groups are unlikely to be due to chance.

Table 2. The differences in NO serum levels based on migraine severity.

Variable	**Migraine severity (MIGSEV Score)**			**p-value**
	Grade 1 (Mild, n=10)	**Grade 2** (Moderate, n=26)	**Grade 3** (Severe, n=15)	
NO serum levels (nmol/ml) Median (min-max)	47.31 (34.85 - 67.15)	88.45 (32.20 -167.45)	96.71 (65.45-169.15)	$p < 0.01$

* Kruskal-Wallis test.

Table 3 presents the results of post-hoc analyses conducted to explore pairwise comparisons of serum Nitric Oxide (NO) levels between the different migraine severity groups. These post-hoc tests were performed after a statistically significant result was obtained from the Kruskal-Wallis test, as shown in Table 2, to determine which specific groups differed from one another. The table provides information on the comparison groups, the statistical test used for the comparison, the resulting p-value, and an interpretation of the statistical significance of the comparison. The first row compares the mild severity group to the moderate severity group. The Mann-Whitney U test was used for this comparison, and the resulting p-value was less than 0.01 ($p < 0.01$). This p-value indicates that there is a statistically significant difference in serum NO levels between the mild and moderate severity groups. The second row compares the mild severity group to the severe severity group. Again, the Mann-Whitney U test was used, and the p-value was less than 0.01 ($p < 0.01$). This result shows a statistically significant difference in serum NO levels between the mild and severe severity groups. The third row compares the moderate severity group to the severe severity group. The Mann-Whitney U test was used, and the p-value was 0.265. This p-value is greater than the conventional significance level of 0.05, indicating that there is no statistically significant difference in serum NO levels between the moderate and severe severity groups.

Table 3. Post-hoc pairwise comparisons of serum NO levels between migraine severity groups.

Comparison groups	**Statistical test used**	**p-value**	**Statistical significance**
Mild Severity vs. Moderate Severity	Mann-Whitney U test	< 0.01	Significant
Mild Severity vs. Severe Severity	Mann-Whitney U test	< 0.01	Significant
Moderate Severity vs. Severe Severity	Mann-Whitney U test	0.265	Not Significant

4. Discussion

The detailed examination of the demographic and clinical characteristics of the study population is crucial for contextualizing the findings related to Nitric Oxide (NO) levels and migraine severity. This section delves into the specific attributes of the 51 female chronic migraineurs who participated in the research, providing a comprehensive overview of their age, body mass index (BMI), education level, marital status, and key migraine-related clinical features. The study cohort was notably defined by its homogeneity in terms of gender, consisting exclusively of women. This focus on female participants is particularly relevant given the well-established gender disparity in migraine prevalence. Migraine disproportionately affects women, especially during their reproductive years. The median age of the participants in this study was 33 years, with the age range spanning from 18 to 50 years. This age range is clinically significant as it largely encompasses the peak years of migraine prevalence in women. The observed pattern of migraine prevalence across the female lifespan, with an increase following puberty, a peak during the reproductive period, and a decline after menopause, has been consistently documented in epidemiological studies. Hormonal fluctuations, particularly those involving estrogen, are frequently cited as a primary contributing factor to this gender difference in migraine. Estrogen exerts a complex and multifaceted influence on various physiological systems that are critical to pain processing and migraine pathophysiology. These include the modulation of neurotransmitter systems, the regulation of neuronal excitability, and the control of vascular tone. Estrogen has been shown to interact with key neurotransmitters such as serotonin, glutamate, Gamma-Aminobutyric Acid (GABA), and norepinephrine. These neurotransmitters play vital roles in the transmission and modulation of pain signals within the central nervous system. Estrogen's influence on these neurotransmitter systems can alter pain sensitivity and the likelihood of experiencing migraine attacks. Furthermore, estrogen affects neuronal excitability in crucial pain-processing regions of the brain, most notably the trigeminal nucleus caudalis (TNC). The TNC is a key relay station for trigeminal sensory information, including pain signals from the head and face. Fluctuations in estrogen levels can alter the responsiveness of neurons within the TNC, thereby influencing migraine susceptibility. Estrogen also plays a role in the production and release of Calcitonin Gene-Related Peptide (CGRP), a potent vasodilator and a key neuropeptide involved in the pathophysiology of migraine. CGRP contributes to pain transmission and the cascade of events leading to migraine headache. Estrogen can modulate CGRP levels and its effects on cerebral blood vessels, thereby impacting migraine development and progression. In addition to its effects on neurotransmitters and neuronal excitability, estrogen influences vascular tone. It partly achieves this through the enhancement of endothelial Nitric Oxide Synthase (eNOS) activity, which is the enzyme responsible for the synthesis of Nitric Oxide (NO). NO, as discussed in the broader context of this study, is a signaling molecule with potent vasodilatory properties and a significant role in neurogenic inflammation, both of which are central to migraine pathophysiology. By focusing exclusively on female participants, this study design enhances the homogeneity of the sample regarding hormonal influences. This reduction in variability allows for a more precise examination of the relationship between NO levels and migraine severity, minimizing the potential confounding effects of hormonal fluctuations. However, it is crucial to acknowledge that this deliberate choice also imposes a limitation on the generalizability of the findings. The results of this study, while informative for understanding migraine in women, cannot be directly extrapolated to male migraineurs. Migraine in men may involve distinct pathophysiological mechanisms and exhibit different patterns of NO metabolism and clinical presentation. Future research should investigate these differences to provide a more comprehensive understanding of migraine across genders. The observed median age of 33 years in this

study population is consistent with findings from previous research conducted in diverse populations. This consistency strengthens the representativeness of the current cohort and supports the notion that migraine prevalence peaks during the reproductive years, regardless of geographical or ethnic variations. The mean Body Mass Index (BMI) of the participants in this study was 23.19 ± 2.97 kg/m². This average BMI falls within the normal weight range, according to standard BMI classifications. This finding is noteworthy because it contrasts with the results of some other studies that have reported higher BMI averages or a greater prevalence of overweight and obesity among individuals with chronic migraine. Several meta-analyses and individual studies have explored the potential association between increased BMI, particularly overweight and obesity, and various aspects of migraine, including migraine risk, frequency, and severity. These studies have suggested that higher BMI may be a contributing factor to the development of migraine, as well as an exacerbating factor for existing migraine conditions. However, the current study's cohort, on average, did not reflect this trend. The participants, as a group, did not exhibit a higher prevalence of overweight or obesity. This discrepancy could potentially be attributed to a variety of factors. It is possible that local population characteristics in Padang City, West Sumatra, may differ from those of the populations included in the studies that reported a link between higher BMI and migraine. Genetic, dietary, and lifestyle variations could contribute to these differences. Another possible explanation for the lower average BMI in this study cohort could be the implementation of stricter exclusion criteria. The study design involved excluding individuals with specific metabolic comorbidities, such as diabetes mellitus and cardiovascular diseases. These conditions are often associated with higher BMI. By excluding individuals with these comorbidities, the study may have inadvertently selected for a group of participants with a lower average BMI. It is important to acknowledge that the potential link between adiposity, associated low-grade inflammation, altered adipokine profiles, and migraine pathophysiology is a complex and evolving area of research. Adipose tissue, or body fat, is not merely an inert storage depot for energy but an active endocrine organ that secretes various signaling molecules, including adipokines. Adipokines can influence inflammatory processes, metabolic function, and vascular health, all of which have been implicated in migraine. However, while the potential role of adiposity in migraine pathophysiology is an important area of investigation, it was not the primary focus of the current study. The study's main objective was to examine the relationship between NO levels and migraine severity. Further research is needed to fully elucidate the complex interplay between adiposity, inflammation, adipokines, NO, and migraine. The sociodemographic findings of this study, including the prevalence of marriage and higher education levels, were generally comparable to reports from other studies conducted in both Western and Asian populations. Specifically, the data revealed that 66.7% of the participants were married, and 68.6% had attained a Diploma or Bachelor's Degree level of education. The observation that a significant proportion of the participants were married aligns with general demographic trends in many societies. Marriage, as a social structure, can influence various lifestyle factors, including social support, stress levels, and daily routines, all of which can potentially impact migraine. Similarly, the finding that a substantial proportion of the participants had higher education levels is consistent with trends of increasing educational attainment globally. Education level can be a marker of socioeconomic status, which, in turn, can influence lifestyle factors such as occupational demands, work-related stress, and sleep patterns. These factors have all been implicated in the development and exacerbation of migraine. It is important to note that while these sociodemographic factors were recorded in this study, their potential influence on migraine was not directly assessed. The study did not specifically investigate how marital status or education level might correlate with migraine

severity or NO levels. However, it is plausible that these factors could indirectly contribute to migraine through their effects on lifestyle, stress, and other related variables. The predominance of migraine without aura in this chronic migraine cohort is consistent with general epidemiological findings. Migraine without aura is the most common subtype of migraine, accounting for a large majority of migraine cases. The high prevalence of migraine without aura in this study population aligns with this broader epidemiological trend. The study also revealed a high rate of positive family history of migraine, with 74.5% of participants reporting a family history of the condition. This finding underscores the significant role of genetic predisposition in migraine susceptibility. Migraine is a complex neurological disorder with a strong genetic component. Individuals with a family history of migraine are at a significantly increased risk of developing the condition themselves. However, it is crucial to acknowledge that the specific genetic factors that influence the chronification of migraine, the transition from episodic to chronic migraine, remain complex and are not fully understood. Migraine chronification is likely a polygenic process, involving the interaction of multiple genes, each contributing a small effect, rather than a single causative gene. Further research is needed to identify the specific genes and genetic pathways involved in migraine chronification. The median attack frequency observed in this study was 16 days per month, with a range from 15 to 24 days per month. The median duration of a typical migraine attack was 6 hours, with a range from 4 to 12 hours. These values are generally within the expected range for individuals with chronic migraine. Chronic migraine is characterized by a high frequency of headache days, and the attack duration observed in this study is consistent with the typical duration of migraine episodes. However, it is worth noting that the median attack frequency observed in this study was lower than that reported in some other studies, such as those conducted in Iran and the United States. These variations in attack frequency across different studies may reflect differences in the study populations, including genetic, environmental, and lifestyle factors. They could also potentially reflect variations in access to healthcare resources and the availability of effective migraine treatments. Differences in healthcare systems and treatment availability can influence the management of migraine and, consequently, the frequency and severity of attacks.[11-14]

The central finding of this study, which highlights increased ictal NO levels correlating with migraine severity, aligns with the known biological actions of NO within the trigeminovascular system. During a migraine attack, elevated NO could amplify vasodilation, increase plasma protein extravasation, and contribute to neurogenic inflammation. These processes are thought to contribute significantly to the intensity and duration of migraine pain. These findings are consistent with previous studies that have reported higher NO levels in migraineurs compared to control subjects, particularly a study that found higher levels in chronic migraineurs compared to episodic migraineurs. However, it is important to acknowledge that direct comparisons between studies are complicated by methodological variations. These variations include differences in study populations, such as chronic versus episodic migraineurs, and those with or without aura. The timing of blood sampling, whether it's during the ictal phase (during an attack) or the interictal phase (between attacks), also significantly influences the results. Some studies that sampled NO levels during the interictal phase found no difference in NO levels compared to controls, suggesting that NO levels might fluctuate significantly, peaking specifically during attacks. The ictal sampling approach used in the current study likely captured this peak activity of NO, providing a more accurate representation of its role in acute migraine attacks. The specific NO levels observed in this study, with a median of 74.8 nmol/ml, require careful comparison with other studies. This is due to potential differences in assay methods and the units used to measure NO (e.g., nmol/ml vs μmol/L vs pg/ml) across different research. For example, the study reported reference

ranges for NO levels in healthy Iranian women (e.g., 10.9-73.7 μmol/L for women aged 30-40). When potentially converted to comparable units, the NO levels observed in the current study might fall within or even above typical ranges, further supporting the notion of elevated NO in chronic migraine patients, particularly during attacks.[15-17]

The link between NO and migraine severity might also involve its complex interplay with oxidative stress and mitochondrial function, which are areas of growing interest in migraine pathophysiology. Cortical Spreading Depression (CSD), a phenomenon that is considered a potential trigger for migraine attacks, increases metabolic demand in the brain. This increased demand can lead to relative hypoxia, disrupting the mitochondrial electron transport chain (ETC) function. The disruption of the ETC results in reduced ATP synthesis, which is the primary energy source for cells, and an increase in the production of reactive oxygen species (ROS). Oxidative stress occurs when there is an imbalance between the production of ROS and the body's antioxidant defenses, leading to potential cellular damage. CSD also triggers the release of NO. Elevated NO can further impair mitochondrial respiration, exacerbating the energy deficit. Additionally, both NO and ROS can activate TRPA1 channels on trigeminal neurons, potentiating the release of CGRP and neuroinflammation in a positive feedback loop, further contributing to migraine pain and its associated symptoms. Therefore, the higher NO levels observed in more severe migraine attacks might reflect a greater degree of underlying neurovascular activation, mitochondrial dysfunction, and oxidative stress. This interpretation aligns with an indirect finding from a clinical trial where Coenzyme Q10 (CoQ10), an antioxidant and ETC cofactor, was shown to reduce both NO levels and migraine severity. This suggests that therapeutic interventions targeting pathways intertwined with NO, such as oxidative stress or mitochondrial bioenergetics, could be relevant for migraine management.[18-20]

5. Conclusion

This study provides compelling evidence for a positive association between serum Nitric Oxide (NO) levels and the severity of chronic migraine in women. The findings indicate that higher NO levels measured during migraine attacks correlate with increased migraine severity. This suggests that NO plays a significant role in the pathophysiology of migraine intensity. It may also serve as a potential biomarker reflecting the clinical burden of chronic migraine. The study's focus on female chronic migraineurs enhances the understanding of migraine mechanisms in this population, which is disproportionately affected by the condition. The results align with previous research implicating NO in key migraine processes such as vasodilation and neurogenic inflammation. The observed correlation between elevated NO levels and greater migraine severity may reflect a higher degree of neurovascular activation, mitochondrial dysfunction, and oxidative stress in more severe attacks. While the study contributes valuable insights, it is important to acknowledge that it is limited to a specific demographic (women) and was conducted in a single location. Future research should aim to replicate these findings in more diverse populations and explore the complex interplay between NO, oxidative stress, mitochondrial function, and other factors involved in migraine pathophysiology. Further investigation into therapeutic interventions targeting NO pathways may also offer new avenues for migraine management.

6. References

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